

-continued

Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys
305					310					315					320
<hr/>															
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu
				325					330					335	
<hr/>															
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr
			340					345					350		
<hr/>															
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu
		355					360					365			
<hr/>															
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp
	370					375					380				
<hr/>															
Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val
385				390						395					400
<hr/>															
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp
				405					410					415	
<hr/>															
Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His
		420						425					430		
<hr/>															
Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro
		435					440					445			

Gly

What is claimed is:

1. A method for purifying a polypeptide from a composition comprising the polypeptide and contaminants, which method comprises the sequential steps of:

- (a) loading the composition onto an ion exchange resin with an equilibration buffer having a first salt concentration;
- (b) washing the ion exchange resin with a wash buffer until a predetermined protein concentration is measured in the flowthrough, wherein the salt concentration of the wash buffer increases from an initial, second salt concentration that is greater than the salt concentration of the equilibration buffer, to a final, third salt concentration;
- (c) passing a fixed volume of wash buffer at the final, third salt concentration over the cation exchange resin; and
- (d) eluting the polypeptide from the ion exchange resin with elution buffer that has a salt concentration that is greater than the final salt concentration of the wash buffer.

2. The method of claim 1 wherein the ion exchange resin is an anion exchange resin.

3. The method of claim 1 wherein the ion exchange resin is a cation exchange resin.

4. The method of claim 3 wherein the cation exchange resin comprises sulphopropyl immobilized on agarose.

5. The method of claim 1 wherein the elution buffer has a higher conductivity than the equilibration buffer.

6. The method of claim 1 wherein the elution buffer comprises about 145 mM Na/HOAc and the equilibration buffer comprises about 70 mM Na/HOAc.

7. The method of claim 1 wherein the elution buffer comprises about 100 mM NaCl and the equilibration buffer comprises about 45 mM NaCl.

8. The method of claim 1 wherein the wash buffer comprises a mixture of equilibration buffer and elution buffer.

9. The method of claim 8 wherein the increase in the salt concentration of the wash buffer during step (b) is achieved by increasing the proportion of elution buffer in the wash buffer.

10. The method of claim 9 wherein the proportion of elution buffer in the wash buffer increases at a constant rate.

11. The method of claim 10 wherein the increase in the proportion of elution buffer causes the salt concentration of the wash buffer to increase at a constant rate of from about 1 mM to about 3 mM per column volume of wash buffer.

12. The method of claim 9 wherein the percentage of elution buffer in the wash buffer increases at two or more different rates during the course of washing in step (b).

13. The method of claim 12 wherein the percentage of elution buffer in the wash buffer increases at a first rate for a first segment of the washing, at a second rate for a second segment of the washing and at a third rate for a third segment of the washing.

14. The method of claim 1 wherein the polypeptide is an antibody.

15. The method of claim 14 wherein the antibody binds HER2.

16. The method of claim 14 wherein the contaminant is a deamidated variant of the antibody.

17. The method of claim 14 wherein the amount of antibody in the composition loaded onto the ion exchange resin is from about 15 mg to about 45 mg per mL of cation exchange resin.

18. The method of claim 1 wherein the predetermined protein concentration in step (b) corresponds to an OD of 0.6 measured at 280 nm.

19. The method of claim 1 wherein from about 0.4 to about 1 column volumes of wash buffer are passed over the ion exchange resin in step (c).

20. The method of claim 1 wherein the pH of the equilibration buffer, wash buffer and elution buffer is approximately the same.

21. The method of claim 1 wherein the pH of the equilibration buffer, wash buffer and elution buffer is approximately 5.5.

22. The method of claim 1 further comprising subjecting the composition comprising the polypeptide to one or more further purification steps either before, during, or after steps